

DAHLIA SUBLIGNOSA (ASTERACEAE):
A SPECIES IN ITS OWN RIGHT

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ABSTRACT

Dahlia dissecta var. *sublignosa* was recognized in 1969 based on available herbarium specimens. Here we present molecular evidence to demonstrate that var. *sublignosa* is not the sister taxon to *D. dissecta* var. *dissecta*, nor is it conspecific with either of its two closest allies, *D. linearis* and *D. foeniculifolia*. Therefore, we elevate *Dahlia dissecta* var. *sublignosa* to the rank of species, as **D. sublignosa** (P.D. Sørensen) D.E. Saar & P.D. Sørensen comb. & stat. nov.

RESUMEN

Dahlia dissecta var. *sublignosa* se reconoció en 1969 basándose en los especímenes de herbario disponibles. Presentamos aquí una prueba molecular para demostrar que var. *sublignosa* no es el taxon hermano de *D. dissecta* var. *dissecta*, ni es conspecifico con ninguno de sus dos semejantes más próximos, *D. linearis* y *D. foeniculifolia*. Por ello, elevamos *Dahlia dissecta* var. *sublignosa* al rango de especie, así como **D. sublignosa** (P.D. Sørensen) D.E. Saar & P.D. Sørensen comb. & stat. nov.

INTRODUCTION

Dahlia dissecta S. Watson presently has two recognized intraspecific taxa: *D. dissecta* var. *dissecta* and *D. dissecta* var. *sublignosa* P.D. Sørensen. *Dahlia dissecta* is in sect. *Entemophyllon*, which includes five other species. *Dahlia dissecta* var. *dissecta* is known from rocky slopes and ledges in the Mexican states of Hidalgo and San Luis Potosí, at elevations of 1900–2500 m. While not common, plants are not difficult to find in these areas. Variety *sublignosa* is known only from the type locality and a nearby location in western Tamaulipas, Mexico, at elevations of 2100–2500 m. The ranges for these two varieties are separated by about 137 km at their closest point, and var. *sublignosa* is not sympatric with any other taxa in sect. *Entemophyllon*.

Dahlia dissecta var. *dissecta* was conservatively described based on limited herbarium specimens known at the time (Sørensen 1969). Morphologically, the two taxa are very similar, at least superficially. Both have ultimate leaf segments that closely resemble each other in shape. All of the species in sect. *Entemophyllon* described at the time had substantial perennating stems except *D. dissecta*, which is wholly herbaceous. Variety *sublignosa* is minimally woody at the base of the canes.

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The varieties differ in that *D. dissecta* var. *sublignosa* tends to be larger, reaching heights of 7–9 dm compared to 3–7.6 dm for var. *dissecta*. Stems of the current year's growth arise directly from a crown of tubers or rootstock on var. *dissecta*, whereas stems of var. *sublignosa* frequently arise from short (5–15 cm) ligneous portions of the previous year's growth. The leaves of var. *sublignosa* can be a little longer (11–19 vs. 10–15 cm), with sometimes smaller ultimate segments (0.5–5.5 vs. 2–9 mm). Outer involucral bracts are 2.5–5.5 mm in width for var. *sublignosa* and 1.8–4 mm for var. *dissecta* (Sørensen 1969). Perhaps the most distinctive feature of difference is the squarish, cusp-tipped leaf segments of var. *sublignosa*, as compared to more rounded leaf segments on var. *dissecta*, which may or may not have a smaller cusp (see Fig. 1a–b).

Observations made with recently collected live material suggested more differences between the varieties than can be seen with herbarium specimens alone. Therefore, a molecular analysis was conducted to clarify the relationship of these two presumed intraspecific taxa.

MATERIALS AND METHODS

Live plant material was obtained from Plant Delights Nursery, Inc., Raleigh, NC, which originated as seed from *Yucca* Do Nursery, Hempstead, TX, accession number D07-615, collected in the Cerro Peña Nevada Nevada Mountains, in the vicinity of the type locality. *D.E. Saar* 3521, 3522 (MUR).

Plants of *Dahlia dissecta* var. *sublignosa* flowered in an outdoor plot, which provided material for chromosome counts from pollen mother cells in developing capitula. Heads of appropriate size were fixed in modified Carnoy's solution (4:3:1 v/v of chloroform: absolute or 95% ethanol: glacial acetic acid), transferred to 70% alcohol, and stored at 4°C until the chromosomes were counted. The staining procedure is summarized in Saar (1999).

Leaf material was collected from two greenhouse plants of *Dahlia dissecta* var. *sublignosa* and one plant of *Dahlia foeniculifolia* Sherff at Murray State University. DNA was extracted using a DNeasy® Plant Mini Kit (Qiagen no. 69104). The internal transcribed spacer regions (ITS) of nuclear ribosomal DNA were amplified using forward primer ITS5m (Sang et al. 1995) to prevent accidental amplification of endophytic fungi, if present (Saar et al. 2001), and reverse primer ITS4 (White et al. 1990). Reactions were in 50 µL volumes and contained 2 units of *Taq* polymerase (Promega, Madison, WI), 0.2 mM of each dNTP, 0.1 µM of each primer, and 75 ng of template. Amplification was carried out on an MJ Research thermal cycler PTC-200 using the following protocol: one cycle of 2 min 30 sec at 95°C, 30 sec at 50°C, and one min at 72°C.; followed by 30 cycles of 30 sec at 95°C, one min at 50°C, and one min at 72°C; and finished with 6 min at 72°C. Amplified products were run on 1.0% agarose gels using 0.5 × TBE and detected with ethidium-bromide fluorescence on a UV transilluminator. Products were cleaned for sequencing using Microcon® Centrifugal Filter

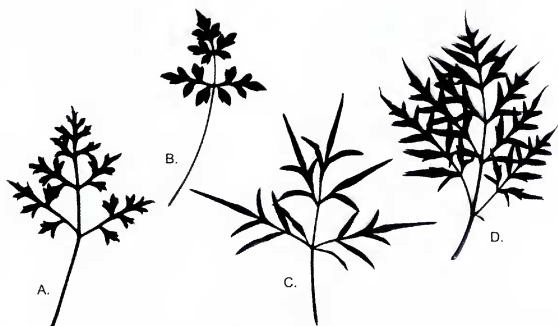


FIG. 1. Comparative leaf shapes from greenhouse-grown plants: A. *Dahlia sublignosa* (formerly *D. dissecta* var. *sublignosa*); B. *Dahlia dissecta* (var. *dissecta*); C. *Dahlia linearis*; D. *Dahlia foeniculifolia*. Leaves from these greenhouse plants are representative of wild-grown plants in general shape but are smaller in size and not proportional to each other as suggested here. See text for leaf dimensions of wild-collected plants.

Devices (YM-100) from Millipore (Bedford, MA). Sequencing was on a Beckman-Coulter capillary sequencer. This procedure was performed twice for var. *sublignosa*, beginning with the DNA extraction from fresh leaves. Our sequence for *D. dissecta* var. *dissecta* (Saar et al. 2003) is congruent with the sequence of var. *dissecta* obtained by Gatt et al. (2000), so repeating the procedure for this taxon was deemed unnecessary.

ITS sequences from three species in sect. *Entemophyllon* were downloaded from GenBank (Saar et al. 2003). Material from the remaining species in the section, *Dahlia congestifolia* P.D. Sørensen, was not available, as it is known only from the type specimen. During field work in 1995, we twice searched the W-facing slopes of the somewhat isolated, small limestone mountain of Cerro Chulco near Apan in extreme southern Hidalgo, the type locality of *D. congestifolia*, but failed to relocate this species. Two other unsuccessful searches were made subsequent to the collection of the type but prior to its formal recognition (Sørensen 1987). *Dahlia merckii* Lehm. was used as the outgroup taxon based on Saar et al. (2003) and its sequence was also obtained from GenBank (Table 1).

Sequences were aligned with Clustal W software (alignment available on request). No gaps were needed to align the ingroup taxa; three one-base gaps were required to align the ingroup with the outgroup taxon. They were ignored in the analysis. Single base polymorphisms are limited to one (r) in *Dahlia*

TABLE 1. GenBank Accession Numbers.

Species	GenBank Accession No.	Source
<i>Dahlia dissecta</i> S. Watson	AY117465	Saar et al. 2003
<i>Dahlia foeniculifolia</i> Sherff	AY117466	Saar et al. 2003
<i>Dahlia linearis</i> Sherff	AY117467	Saar et al. 2003
<i>Dahlia merckii</i> Lehm.	AY117471	Saar et al. 2003
<i>Dahlia rupicola</i> P.D. Sørensen	AY117468	Saar et al. 2003
<i>Dahlia scapigeroides</i> Sherff	AY117469	Saar et al. 2003
<i>Dahlia subligiosa</i> (P.D. Sørensen) Saar & P.D. Sørensen	DQ198259	this study

linearis Sherff and two (*y*, *s*) in *D. scapigeroides* Sherff, which were included in the matrix format symbols for analysis. A branch-and-bound search was performed using PAUP* 4.0v8 (Swofford 1998) on a Macintosh G5 computer. Bootstrap analysis (Felsenstein 1985) was performed using 1000 replicates. Pairwise distances [uncorrected ("p") distance matrix] were calculated with PAUP.

RESULTS

The ITS sequences for both plants of *Dahlia dissecta* var. *subligiosa* are identical, so only one sequence was submitted to GenBank. The new sequence obtained for *D. foeniculifolia* is consistent with that of Saar et al. (2003) but is of better quality (no unknowns or polymorphisms), presumably due to the availability of better leaf material from the same plant (GenBank sequence updated).

The phylogenetic analysis produced two trees of a shortest length of 70 steps (CI 0.971, RI 0.917, RC 0.890, HI 0.029). A total of 671 base pairs were aligned and analyzed: 251 bp in ITS-1, 220 bp in ITS-2, and the remaining bp from flanking regions of coding nrDNA.

Results show that the sister taxon to *Dahlia dissecta* var. *subligiosa* is not var. *dissecta* but either *D. linearis* or *D. foeniculifolia*. The tree shown in Figure 2, based on a strict consensus, results in a polytomy between *D. linearis*, *D. foeniculifolia*, and the two samples of var. *subligiosa*.

A distance matrix of sect. *Entemophyllon* has an average of 1.54% ($\delta = 0.0066$) sequence divergence among the six taxa. *Dahlia dissecta* var. *subligiosa* differs from *D. linearis* by 0.91% and from *D. foeniculifolia* by 1.21%.

The chromosome number is $n=17$.

DISCUSSION

The phylogenetic analysis shows that *Dahlia dissecta* var. *dissecta* and var. *subligiosa* are not conspecific. Leaf and flower morphology and ITS sequences also do not suggest that it is a variety of the next closest taxa, *D. linearis* or *D. foeniculifolia* (Fig. 1). The sequence divergences between var. *subligiosa* and *D.*

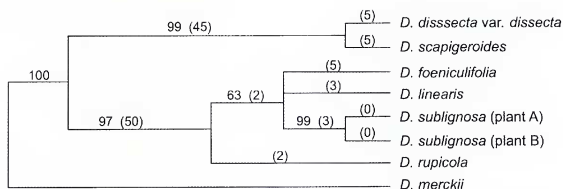


FIG. 2. Phylogenetic analysis of *Dahlia* sect. *Entemophyllon* using ITS sequences. Outgroup is *D. merckii* (not in sect. *Entemophyllon*). Numbers above branches represent bootstrap values; numbers in parenthesis indicate number of changes.

linearis and between var. *sublignosa* and *D. foeniculifolia* (0.91 and 1.21%, respectively) are consistent with the other species in the section and other clades in the genus. By comparison, the "variable root clade" (Saar et al. 2003) averages 0.97% ($\delta = 0.0087$) divergence for 10 taxa and the "core *Dahlia* clade" (Saar et al. 2003) averages 0.87% ($\delta = 0.0096$) over 15 taxa. Therefore, it is concluded that *D. dissecta* var. *sublignosa* should be elevated to the rank of species, coordinate with the other taxa of the section:

Dahlia sublignosa (P.D. Sørensen) D.E. Saar & P.D. Sørensen, comb. & stat. nov. (Fig. 3). TYPE: MEXICO, TAMAULIPAS. 4.8 km N of Miquihuana in forest dominated by *Pinus* (99°47'N Lat; 23°36'W Long), elev. ca. 2100 m, 14 Jul 1949, Stanford, Taylor, & Lauber 2436 (HOLOTYPE: NY; ISOTYPES: GH, MICH, TEX, UC, US-2, WTU).

Dahlia sublignosa is readily distinguished from either *D. linearis* or *D. foeniculifolia* by its shorter ultimate leaf segments (0.5–5.5 mm vs. 9–23 for *D. linearis* and 30–55 mm for *D. foeniculifolia*). The chromosome number of $n=17$ is consistent with five of the other species in the section; the number is not known for the sixth species, *Dahlia congestifolia*.

The elevation of *Dahlia sublignosa* to rank of species brings the number of "wild" species in the genus to 36, but does not include the cultivated forms often called *D. variabilis* Desf. or occasionally *D. pinnata* Cav, but see Hansen and Hjerting (1996) for clarification of the latter binomial.

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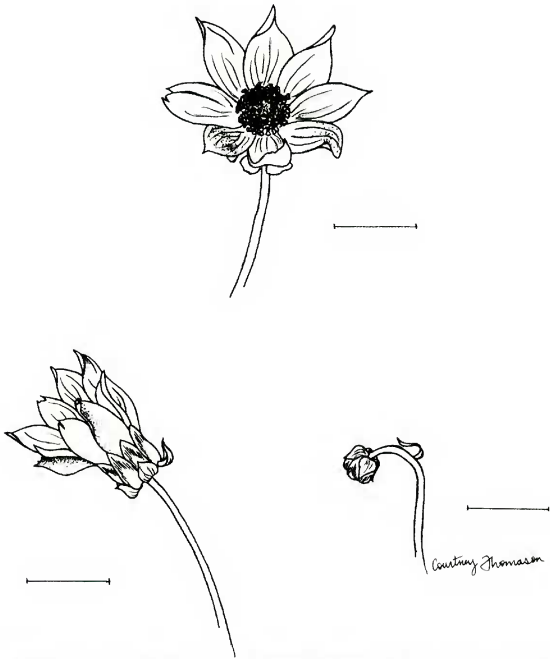


FIG. 3. Flower head of *Dahlia sublignosa*. Scale bar represents 1 cm.

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